DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Cypermethrin PC Code No.: 109702

2. **TEST MATERIAL:** Cypermethrin Technical 40/60 Purity: 40.6% cis/59.4% trans

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Cypermethrin Applied to Formulated Sediment Under Static-Renewal Conditions.
Study Completion Date:	May 7, 2009
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571
Sponsor:	Pyrethroid Working Group Beverage & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6129
MRID No.:	47946602
DP Barcode:	420006

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 06/08/10

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 06/10/10

5. **APPROVED BY:** Stephen Carey, Biologist, OCSPP/EFED/ERB6

Signature: Stephen Carey **Date:** 7/21/15

6. **STUDY PARAMETERS:**

Age of Test Organism:	7 to 8 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 5.5 µg a.i./kg 95% C.I.: 4.0 to 8.6 µg a.i./kg
Slope: 3.65 (1.73 to 5.56)
NOAEC: 2.7 µg a.i./kg
LOAEC: 5.3 µg a.i./kg

Growth:
EC_{50}: 4.7 µg a.i./kg 95% C.I.: 3.2 to 6.9 µg a.i./kg
Slope: 3.57±1.28
NOAEC: <1.1 µg a.i./kg
LOAEC: 1.1 µg a.i./kg

Based upon ESTIMATED[1] pore water concentrations:

Survival:
LC_{50}: 0.002 µg a.i./L 95% C.I.: 0.0016 to 0.003 µg a.i./L
Slope: 3.65 (1.73 to 5.56)
NOAEC: 0.001 µg a.i./L
LOAEC: 0.002 µg a.i./L

Growth (dry weight):
IC_{50}: 0.002 µg a.i./L 95% C.I.: 0.001 to 0.003 µg a.i./L
Slope: 3.57±1.28
NOAEC: <0.0004 µg a.i./L
LOAEC: 0.0004 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 306 µg a.i./kg TOC 95% C.I.: 222 to 478 µg a.i./kg TOC
Slope: 3.65 (1.73 to 5.56)
NOAEC: 150 µg a.i./kg TOC
LOAEC: 294 µg a.i./kg TOC

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1 Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Growth (dry weight):
EC_{50}: 261 µg a.i./kg TOC 95% C.I.: 178 to 383 µg a.i./kg TOC
NOAEC: <61 µg a.i./kg TOC
LOAEC: 61 µg a.i./kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

A NOAEC could not be determined in this study, as there were significant ($p<0.05$) reductions in dry weight of amphipods at all treatment levels ranging from 15 to 46%, relative to the negative control. It should be noted, however, that growth is an optional endpoint according to OCSPP (formerly OPPTS) 850.1735 guidance; survival is the primary endpoint.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Hyalella azteca*
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	7 to 8 days old

Guideline Criteria	Reported Information
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures maintained in *ca.* 15 L of culture water (same source as dilution water) under flow-through conditions.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 8 days prior to test initiation and placed in *ca.* 8 L of water. Juvenile amphipods (<24 hours old) produced by the isolated adults were then transferred to *ca.* 0.80 L of laboratory dilution water and reared under static conditions for 7 to 8 days with gentle aeration. During the holding period, the dissolved oxygen ranged from 7.9 to 8.6 mg/L and temperature ranged from 22 to 24 °C.
Feeding:	During holding and acclimation, amphipods were fed every other day with 2.5 mL of a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) and 2.5 mL of *Ankistrodesmus falcatus*.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 34 to 46 and 17 to 19 mg/L, respectively, a pH range of 6.2 to 7.0, and a specific conductance range of 210 to 250 µmhos/cm. Monthly analysis of the water source indicated a TOC 0.55 mg/L for February 2009. Formulated sediment (Batch No. 101508) was prepared according to OECD Guideline No. 218. The following components were mixed on a dry weight basis: 2.4 kg sphagnum peat, 8.0 kg kaolin clay, and 29.6 kg fine sand.
Does water support test animals without observable signs of stress?	Yes.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. During the study, ammonia levels (as N) in the overlying water were generally ≤0.62 mg/L (one measurement of 3.0 mg/L on Day 10 at the nominal 1.3 µg/kg level).
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 21 to 25°C (see Reviewer's Comments section)
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans*..	6.6 to 7.2
Dissolved Oxygen Maintained between 40 and 100%.	6.0 to 8.4 mg/L (≥70% ASV at 23°C; reviewer-calculated)

Guideline Criteria	Reported Information
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	48 to 56 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	290 to 350 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 79% sand, 6% silt, 15% clay (sandy loam; reviewer-derived from USDA soil texture triangle) Organic carbon content – 1.8% Solids – 71.94% pH – 6.6 Ammonia concentration of pore water – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	None reported
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	<u>Cypermethrin Technical 40/60</u> Synonym: FMC 30980 IUPAC Name: (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS Name: cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate CAS No.: 52315-07-8 Description: not reported Lot No.: PL07-0633 Purity: 40.6% cis-isomer, 59.4% trans-isomer Storage: dark, room temperature

Guideline Criteria	Reported Information
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	Two hundred (200) mL of a 25-µg a.i./L primary stock solution was prepared in acetone. From this, 100 mL of a 2.5 µg a.i./mL secondary stock solution was prepared in acetone. Five individual dosing solutions were prepared using a combination of the primary and secondary stocks, and bringing the mixture to 10 mL with acetone. All stock and dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand in glass Petri dishes, and the solvent was allowed to evaporate off for 35 minutes. The dry sand was then added to 3.0 kg of wet sediment (total of 2.208 kg dw) in individual 1-gallon jars. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 4 ± 2°C during conditioning. The treated sediments were allowed to equilibrate for a 14-day period in the refrigerator. Twice a week during the conditioning period and prior to addition to the exposure vessels (day -1), the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous. The range of concentrations (0.63 to 10 µg a.i./kg) was based upon the results of a preliminary range finding study.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	300-mL glass vessels containing 100 mL (approx. 4.0-cm layer) of sediment (equivalent to 68 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL. Test vessels were covered with 40-mesh Nitex® screen for drainage.
Type of Dilution System Daily renewal or a flow-through system may be used.	Intermittent flow-through

Guideline Criteria	Reported Information
Flow Rate 2 volume changes/day	2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 510 to 880 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 9 mL per 2.208 kg dw sediment. The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was replaced twice daily using an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.	<u>Preliminary toxicity assessment</u> • Treated sediment equilibrated for 10 days • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.010, 0.10, 1.0, 10, and 100 µg a.i./kg • three replicates per level, each containing 10 organisms • Survival averaged 100 (negative control), 97 (solvent control), 100, 100, 100, 3, and 0%, respectively • Dry weight averaged 0.11 (negative control), 0.11 (solvent control), 0.12, 0.09, 0.10, and 0.06 mg, respectively
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for mortality and abnormal behavior.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 0.63, 1.3, 2.5, 5.0, and 10 µg a.i./kg sediment
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level An additional 24 replicates were maintained for chemical analysis
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	1.0 mL of yeast, cereal leaves, and flaked fish food suspension (YCT) once daily.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Overlying water: For all levels, total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample on Days 0 and 10. DO, temperature, and pH were measured in each replicate vessel on Days 0 and 10 and in one alternating replicate from each level on Days 1 to 9. Temperature was also continuously monitored in an auxiliary vessel in the water bath. Pore water: Redox potential, pH, ammonia, and dissolved organic carbon (DOC) were measured on Days 0 and 10.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Surrogate samples vessels were collected on Days 0 and 10, and concentrations of cypermethrin were determined in pore water and sediment (see Reviewer's Comments section). The sediment/pore water matrices were isolated by centrifuging for 15 to 30 minutes at 1200 *g*. Aliquots of the dosing stock solutions were analyzed for cypermethrin. In addition, treated sediment from all levels were analyzed for cypermethrin prior to the allocation of the sediment into the replicate vessels (following equilibration).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: the routine water, sediment, and food contaminant screening analyses.
Control Criteria Was control mortality ≤20%? Were control *C. tentans* an average size of ≥0.6 g?	Mortality: Negative control – 0% Solvent control – 5%
Percent Recovery of Chemical:	Procedural recoveries (QC samples) conducted concurrently with sample analysis: Sediment: 89.2 to 120% of nominal (with one outlier of 156%) Aqueous: 97.9 to 117% of nominal
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	- Survival - Dry weight
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Dry Weight	
Nominal (µg a.i./kg)	Mean Measured[a]						
	Sediment (µg a.i./kg dw)	Pore Water (µg a.i./L)	Overlying Water (µg a.i./L)	Mean %	% Inhibition	mg per larvae	% inhibition
Control	<LOQ	<LOQ	Not assessed	100	N/A	0.13	N/A
S. Control	<LOQ	<LOQ	Not assessed	95	5.0	0.13	0
0.63	1.1	0.021	Not assessed	98	2.0	0.10*	23
1.3	1.8	0.036	Not assessed	95	5.0	0.11	15
2.5	2.7	0.058	Not assessed	93	7.0	0.07*	46
5.0	5.3	0.11	Not assessed	56*	44	0.06[b]	54
10	8.8	0.22	Not assessed	18*	82	0.01[b]	92

[a] LOQ were equivalent to 0.022 to 0.024 µg a.i./kg for sediment samples and 0.0019 to 0.0059 µg a.i./L for pore water samples.
[b] Excluded from statistical analyses due to significant effect on survival.
* Statistically different ($p \leq 0.05$) compared to the negative control.

Other Significant Results:

Biological: After 10 days, survival averaged 100 and 95% for the negative and solvent controls, respectively, and 98, 95, 93, 56, and 18% for the mean-measured 1.1, 1.8, 2.7, 5.3, and 8.8 µg a.i./kg sediment levels, respectively. Differences at the 5.3 and 8.8 µg a.i./kg levels were statistically-reduced ($p \leq 0.05$) compared to the negative control. The 10-day LC_{50} (with 95% C.I.) was reported by the study author to be 5.9 (5.1 to 6.6) µg a.i./kg sediment, and the NOAEC for survival was 2.7 µg a.i./kg.

After 10 days, dry weight averaged 0.13 mg per larvae at both the negative and solvent control levels, and 0.10, 0.11, 0.07, 0.06, and 0.01 mg per larvae at the mean-measured 1.1, 1.8, 2.7, 5.3, and 8.8 µg a.i./kg sediment levels, respectively. Differences at the 1.1 and 2.5 µg a.i./kg sediment levels were statistically-reduced ($p \leq 0.05$) compared to the negative control (the 5.3 and 8.8 µg a.i./kg levels were not statistically compared due to the significant effect on survival at these levels). However, as no statistically-significant reduction in growth was indicated at the 1.8 µg a.i./kg sediment level, the difference observed at the 1.1 µg a.i./kg level was considered by the study author to be incidental to treatment. The 10-day EC_{50} (with 95% C.I.) was reportedly 5.2 (2.6 to 6.5) µg a.i./kg sediment, and the NOAEC for amphipod growth was 1.8 µg a.i./kg.

Analytical: Concentrations of cypermethrin were determined on Days 0 and 10 in sediment and pore water only (see Reviewer's Comments section). Concentrations remained relatively constant in sediment and pore water. On day 10, recoveries in sediment were within ±31% of Day-0 results (reviewer-calculated). Mean-measured sediment concentrations were 1.1, 1.8, 2.7, 5.3, and 8.8 µg a.i./kg sediment, representing 180, 140, 110, 110, and 88% of the nominal treatment levels, respectively. In pore water, concentrations of cypermethrin increased slightly (14 to 39% of Day-0 values; reviewer-calculated) at all but one concentration level (nominal 1.3 µg/kg level; -2.8% of Day-0 value).

Nominal Sediment Concn. (µg a.i./kg)	Sediment, µg a.i./kg		Pore Water, µg a.i./L		Overlying Water	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control	<0.022	<0.024	<0.0019	<0.0059	Not assessed	Not assessed
S. Control	<0.022	<0.024	<0.0020	<0.0052	Not assessed	Not assessed
0.63	1.3	0.90	0.019	0.023	Not assessed	Not assessed
1.3	2.0	1.7	0.036	0.035	Not assessed	Not assessed
2.5	2.4	2.9	0.049	0.068	Not assessed	Not assessed
5.0	6.0	4.6	0.10	0.12	Not assessed	Not assessed
10	8.6	9.0	0.21	0.24	Not assessed	Not assessed

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (dry weight). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. A statistically-significant difference was indicated for survival; however, survival for both the control and solvent control groups was ≥95% and differences observed between the two groups were within the range of natural variability. Control performance for growth was statistically similar. All treatment groups were compared to the negative control data to determine treatment-level effects.

Normality of the data was evaluated using the Chi-Square Test, and homogeneity of variance was evaluated using Bartlett's Test or Hartley's Test. Survival data did not meet the assumption of homogeneity of variance and was therefore analyzed using the non-parametric Steel's Many-One Rank Test at the 95% level of certainty. Growth data met both assumptions and was thus analyzed using Dunnett's Test at the 95% level of certainty. NOAEC and LOAEC values were

assigned based upon significance.

The linear interpolation method was used to calculate the LC/EC_{50} values with associated 95% confidence intervals.

Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

Survival:
LC_{50}: 5.9 µg a.i./kg 95% C.I.: 5.1 to 6.6 µg a.i./kg
NOAEC: 2.7 µg a.i./kg
LOAEC: 5.3 µg a.i./kg

Growth:
EC_{50}: 5.2 µg a.i./kg 95% C.I.: 2.6 to 6.5 µg a.i./kg
NOAEC: 1.8 µg a.i./kg
LOAEC: 2.7 µg a.i./kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed data for day 10 survival and dry weight. For both endpoints the negative and solvent control data were compared using a Student's t-test; for survival, a significant reduction ($p<0.05$; 5%) was detected in the solvent control, relative to the negative control. However, because survival was 100% in the negative control and 95% in the solvent control, solvent interference was not suspected to have played a role in this study. The data for dry weight were further tested using Shapiro-Wilk's test to confirm normality and using Levene's test to confirm homogeneity of variances. The 5.3 and 8.8 µg a.i./kg dry weight data were excluded from this analysis, due to significant effects on survival at these levels. Dry weight data satisfied the assumptions of ANOVA, so the NOAEC and LOAEC were determined using this test, followed by William's test (because of the general trend toward a dose-dependent response). The results of William's test seemed justified given the magnitude of the reductions at all treated levels, relative to the negative control (i.e., 15 to 46%). There was at least one group with zero variance for the survival data, so the NOAEC and LOAEC for this endpoint was determined using the non-parametric Steel's Many-One Rank test. These analyses were conducted using Toxstat 3.5 statistical software. The LC_{50} and EC_{50} values were determined using the Probit method. For survival, the Probit method was run using Toxanal 2009 and selected over the other methods as the best for characterizing the data, despite the poor fit of the data to the model (Goodness of fit probability = 0.02). The Probit method used to obtain the EC_{50} for dry weight was run using Nuthatch statistical software.

All of the above statistical analyses were performed in terms of the mean-measured sediment and estimated pore water treatment concentrations. Sediment endpoints are also calculated on an organic carbon-normalized basis, based on the following equation using an average TOC of 1.8%:

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 5.5 µg a.i./kg 95% C.I.: 4.0 to 8.6 µg a.i./kg
Slope: 3.65 (1.73 to 5.56)
NOAEC: 2.7 µg a.i./kg
LOAEC: 5.3 µg a.i./kg

Growth:
EC_{50}: 4.7 µg a.i./kg 95% C.I.: 3.2 to 6.9 µg a.i./kg
Slope: 3.57±1.28
NOAEC: <1.1 µg a.i./kg
LOAEC: 1.1 µg a.i./kg

Based upon ESTIMATED[1] pore water concentrations:

Survival:
LC_{50}: 0.002 µg a.i./L 95% C.I.: 0.0016 to 0.003 µg a.i./L
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NOAEC: 0.001 µg a.i./L
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Growth (dry weight):
IC_{50}: 0.002 µg a.i./L 95% C.I.: 0.001 to 0.003 µg a.i./L
Slope: 3.57±1.28
NOAEC: <0.0004 µg a.i./L
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Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 306 µg a.i./kg TOC 95% C.I.: 222 to 478 µg a.i./kg TOC
Slope: 3.65 (1.73 to 5.56)
NOAEC: 150 µg a.i./kg TOC
LOAEC: 294 µg a.i./kg TOC

Growth (dry weight):
EC_{50}: 261 µg a.i./kg TOC 95% C.I.: 178 to 383 µg a.i./kg TOC
NOAEC: <61 µg a.i./kg TOC

LOAEC: 61 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOAEC and LOAEC for dry weight differed from the study author's. Both detected significant reductions from the negative control at the lowest treatment level, but the study author dismissed this as treatment-related because Dunnett's test failed to detect a significant reduction at the next higher level (i.e., 1.8 µg a.i./kg). The reviewer maintains that reductions from the negative control ranged from 15 to 46% for all treated levels that did not experience reduced survival and while the response for this endpoint was not linear, it was generally directional and suggestively treatment-related at all test levels. As a result, the reviewer concluded that a NOAEC could not be determined for this study.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized) and estimated pore water concentrations in the Conclusions section of the DER.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

This reviewer notes that the concentration of cypermethrin measured in pore water likely reflects both "freely dissolved" chemical (i.e., chemical that is not sorbed onto particulate organic carbon (POC) or dissolved organic carbon (DOC) in addition to dissolved chemical that is sorbed to DOC. This finding is indicated by the fact that the extraction and analytical methods used in this study do not distinguish among the two phases of chemical (freely dissolved and DOC-sorbed). It is also indicated by the much higher measured concentrations of cypermethrin in pore water than would be expected based on estimated values using sediment cypermethrin concentrations, its Koc, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like cypermethrin, DOC in pore water can substantially reduce its bioavailability and toxicity. It is further noted that the pore water estimated environmental concentrations (EECs) generated using the Agency's PRZM/EXAMS model are based on freely dissolved chemical. Therefore, some downward adjustment of these pore water toxicity values using appropriate methods (e.g., Koc and DOC concentration in pore water) will likely be needed when comparing these values to freely dissolved EECs generated using PRZM/EXAMS. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations.

Instead, this reviewer has estimated freely dissolved pore water endpoints based on measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (1.8%) and the mean K_{OC} (141,700 L/kg-OC, MRID 42129002) for cypermethrin. These estimated pore water endpoints, which are based on the freely dissolved test material (i.e.,

chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 – 328,500 L/kg). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC}. Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

Nominal Sediment (µg a.i./kg)	Mean-measured Sediment (µg a.i./kg)	Estimated Pore Water (µg a.i./L)	OC-Normalized Sediment (µg a.i./g OC)
0.63	1.1	0.0004	61
1.3	1.8	0.0007	100
2.5	2.7	0.001	150
5.0	5.3	0.002	294
10	8.8	0.003	489

Analysis of the stock solution samples used to dose the test sediments ranged from 94 to 120% of nominal fortified concentrations. Pretest analysis of the spiked sediment following equilibration and prior to allocation into the replicate exposure vessels ranged from 110 to 160% of nominal concentrations.

It was reported in the protocol deviation section that the solvent control sediment was not prepared at the same time as the test sediments. The solvent control sediment was dosed (with acetone) and allowed to mix/equilibrate for *ca.* 4 days, whereas the other sediments were allowed to mix/equilibrate for *ca.* 14 days. It was reported that this deviation had no significant impact on the results or interpretation of the study since this sediment contained residual solvent only and no test substance.

In pore water (measured at each level on Days 0 and 10), the redox potential ranged from 240 to 290 mV, the pH ranged 6.7 to 6.9, the DOC ranged from 96 to 180 mg C/L, and the ammonia (as N) ranged from 0.82 to 3.6 mg/L.

The analytical method used to quantify cypermethrin in (formulated) sediment was validated in December 2008. Fortified samples were extracted two to three times with methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.100 and 100 µg/kg, recoveries averaged 110 ± 9.29% and 97.3 ± 5.05%, respectively, with a limit of quantitation (LOQ) of 0.0225 µg a.i./kg.

The analytical method used to quantify cypermethrin in freshwater was validated in January 2009. Fortified samples were acidified and extracted twice with ethyl acetate; the combined extracts were reduced in volume using rotary evaporation (30°C) and taken to dryness under nitrogen

(room temperature). The residues were re-constituted in 0.1% peanut oil in acetone and analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.00100 (sample LOQ), 0.00300, 0.0200, and 0.0500 µg/L, recoveries averaged 114 ± 3.82%. Due to the low concentrations being tested, the LOQ was set at 0.00100 µg/L; sample LOQ recoveries averaged 110 ± 16.1%.

A discrepancy was noted regarding continuous temperature monitoring. It was reported in paragraph 1 on page 27 of the study report that this temperature range was 21 to 25°C; however, in the footnote to Table 1 on page 35 of the study report, the continuous-monitoring temperature range was reported as 22 to 24°C.

It was reported that representative samples of the overlying water source were periodically analyzed for pesticides, PCBs, and toxic metals, and that none of these compounds were detected in any of the water samples analyzed in agreement with ASTM guidelines.

Definitive test dates were February 10 to 20, 2009.

This study was submitted to fulfill proposed OPPTS Draft 850.1735, whole sediment acute toxicity to freshwater invertebrates.

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15. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
     ***********************************************************************
     CONC.     NUMBER         NUMBER         PERCENT        BINOMIAL
               EXPOSED        DEAD           DEAD           PROB.(PERCENT)
      8.8       80             66             82.5           0
      5.3       80             35             43.75          0
      2.7       80             6              7.500001       0
      1.8       80             4              5              0
      1.1       80             2              2.5            0

     BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
     CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
     UNRELIABLE.  USE THE INTERVALS CALCULATED BY THE OTHER TESTS.


     AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.721081


     RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
        SPAN      G           LC50           95 PERCENT CONFIDENCE LIMITS
      2         3.326162E-02  5.591582       5.093454       6.178858


     RESULTS CALCULATED USING THE PROBIT METHOD
     ITERATIONS             G               H              GOODNESS OF FIT
PROBABILITY
          4                  .2751254       3.277702
.0200392

     SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
     USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

     SLOPE   =               3.647577
     95 PERCENT CONFIDENCE LIMITS = 1.734335    AND    5.560818

     INTERCEPT=-2.695379

     LC50 =    5.482147
     95 PERCENT CONFIDENCE LIMITS =  4.016779 AND  8.651604

              LC25 =         3.581239
     95 PERCENT CONFIDENCE LIMITS =  2.205044 AND  4.868022

              LC10 =         2.441147
     95 PERCENT CONFIDENCE LIMITS =  1.089244 AND  3.423121

              LC05 =         1.940857
     95 PERCENT CONFIDENCE LIMITS =  .6920657 AND  2.861495
     ***********************************************************************

   Title:  Percent Survival
```

File: 6602s Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	100.0000	Calculated t value =	2.6458
GRP2 (Blank cntl) Mean =	95.0000	Degrees of freedom =	14
Difference in means =	5.0000		

2-sided t value (0.05,14) = 2.1448** Significant difference at alpha=0.05
2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Percent Survival
File: 6602s Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Neg Control	100.0000				
2	1.1	97.5000	60.00	46.00	8.00	
3	1.8	95.0000	52.00	46.00	8.00	
4	2.7	92.5000	52.00	46.00	8.00	
5	5.3	56.2500	36.00	46.00	8.00	*
6	8.8	17.5000	36.00	46.00	8.00	*

Critical values are 1 tailed (k = 5)

Title: Dry Weight
File: 6602w Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.1275	Calculated t value =	-0.0773
GRP2 (Blank cntl) Mean =	0.1288	Degrees of freedom =	14
Difference in means =	-0.0013		

2-sided t value (0.05,14) = 2.1448 No significant difference at alpha=0.05
2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

```
     D =   0.0087
     W =   0.9671

     Critical W  = 0.9040 (alpha = 0.01 , N = 32)
              W  = 0.9300 (alpha = 0.05 , N = 32)
```

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0004	0.0001	1.3349
Within (Error)	28	0.0027	0.0001	
Total	31	0.0031		

(p-value = 0.2829)

Critical F = 4.5681 (alpha = 0.01, df = 3,28)
= 2.9467 (alpha = 0.05, df = 3,28)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0136	0.0045	14.5107
Within (Error)	28	0.0087	0.0003	
Total	31	0.0223		

(p-value = 0.0000)

```
     Critical F  = 4.5681  (alpha = 0.01, df = 3,28)
                 = 2.9467  (alpha = 0.05, df = 3,28)

     Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)
```

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg Control	0.1275	0.1275		
2	1.1	0.1025	0.1025	2.8304	*
3	1.8	0.1125	0.1125	1.6983	
4	2.7	0.0713	0.0713	6.3685	*

Dunnett critical value = 2.1700 (1 Tailed, alpha = 0.05, df [used] = 3,24)
(Actual df = 3,28)

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	8			
2	1.1	8	0.0192	15.0	0.0250
3	1.8	8	0.0192	15.0	0.0150
4	2.7	8	0.0192	15.0	0.0562

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	8	0.1275	0.1275	0.1275

2	1.1	8	0.1025	0.1025	0.1075
3	1.8	8	0.1125	0.1125	0.1075
4	2.7	8	0.0713	0.0713	0.0713

Title: Dry Weight
File: 6602wr Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	0.1275				
1.1	0.1075	2.2644	*	1.7000	k= 1, v=28
1.8	0.1075	2.2644	*	1.7800	k= 2, v=28
2.7	0.0713	6.3685	*	1.8100	k= 3, v=28

s = 0.0177

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

6602W : Dry Weight

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.128	.		
1.1	0.107	1.154	N.S.	
1.8	0.107	1.154	N.S.	
2.7	0.0712	3.245	<0.005	*
5.3	0.065	3.605	<0.005	*
8.8	0.01	5.944	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.6	0.57	4.7	0.23	0.35
EC10	2.1	0.85	5.0	0.19	0.41
EC25	3.1	1.6	5.7	0.14	0.53
EC50	4.7	3.2	6.9	0.083	0.68

Slope = 3.57 Std.Err. = 1.28

Goodness of fit: p = 0.15 based on DF= 3.0 39.

6602W : Dry Weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	0.128	0.114	0.0136	100.	0.00
1.10	8.00	0.102	0.112	-0.00999	98.8	1.20
1.80	8.00	0.113	0.106	0.00634	93.2	6.76
2.70	8.00	0.0712	0.0918	-0.0206	80.7	19.3
5.30	8.00	0.0650	0.0488	0.0162	42.8	57.2
8.80	5.00	0.0100	0.0190	-0.00900	16.7	83.3